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Project No. _____

Book No. _____

TITLE The mutant contd

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Annealing & Synthesis

	(D-A)	(F→Tyr)	Control
Annealing	1.0μl 2894	1.0μl 2904	1.0μl
	1.0μl Ann. Buff	1.0μl Ann. Buff	1.0μl
	5.0μl ssDNA	5μl ssDNA	5.0μl
	3.0μl H ₂ O	3.0μl H ₂ O	3.0μl

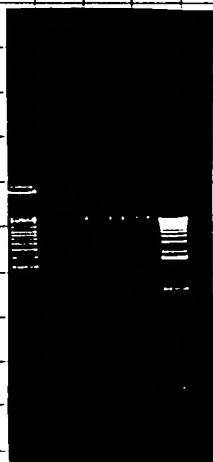
75°C for 2min → quick chill in ice water → ice

Synthesis Add 0.25μl T7 Pol (sequase) →
0.5μl T4 ligase (LTI) →

5' on ice, 5' at room temp 35min at 37°C

Add 90μl (10mMEDTA + 10mM TRIS) → ice.

Load 15μl in agarose gel.



The synthesis looks alright. But I have some concern because the reaction did produce clear cut band.

Anyway proceed with the transformation dil sample (1:25).

DH5α F' 2Q 100μl
2μl 2894 or 2904 or control

25' on ice, 40' at 42°C → 30Sec. on ice

Overlay with top agar (10μl and 90μl sample)
37°C 0/15

T Pag N _____

Witnessed & Understood by me,

[Signature]

Date

4/6/95

Invented by

R C [Signature]

Date

3/29/95